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# Home Cage Compared with Induction Chamber for Euthanasia of Laboratory Rats

**Debra L Hickman**

**This study compared behavioral and physiologic changes in Sprague–Dawley and Brown Norway rats that were euthanized by using a 30% volume displacement rate of $CO_2$ in either their home cage or an induction chamber; rats euthanized in the home cage were hypothesized to demonstrate a higher level of animal wellbeing. No significant differences were detected in the physiologic responses to home cage versus induction chamber euthanasia groups. A few strain-related behavioral differences occurred. The number of digs per second was higher in Brown Norway compared with Sprague–Dawley rats when in the home cage, where a digging substrate was present. Rearing frequency was higher in both Brown Norway and Sprague–Dawley rats in the induction chamber compared with the home cage. This study demonstrated that although strain-specific differences were associated with the process of euthanasia, there were no significant differences between the treatment groups of home cage compared with induction chamber. This finding suggests that—from the perspective of a rat—either the home cage or an induction chamber can be used for euthanasia, with likely extension of this conclusion to use of either method to the induction of anesthesia.**

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Global standards expect that steps will be taken to minimize the potential for pain and distress for animals when they are euthanized.[2,6,13] The numerous recommendations for the process of euthanizing rodents used in research include the maintenance of stable groups, minimizing transport prior to the euthanasia process, and selection of euthanasia methods that minimize pain and distress. An additional recommendation is that the wellbeing of rodents is improved when the rodent is euthanized within its home cage.[1,2] This recommendation is based on a large body of literature that demonstrates transient increases in blood pressure, heart rate, and corticosterone after a cage change with the associated alterations of scent-marking.[8,15,21,24-26] But to date, few studies have evaluated the use of an induction chamber as compared with a home cage. Recently, one laboratory reported that the presence or absence of home-cage bedding in an induction chamber used to anesthetize mice with isoflurane did not significantly affect serum corticosterone levels in mice.[20]

This question is a critical one because of an apparent professional dichotomy in best practices for the improvement of animal wellbeing in euthanasia and anesthesia. Although a standard practice is to use the home cage during the delivery of an inhaled anesthetic for euthanasia, prevalent standard laboratory practice supports the use of an induction chamber for preprocedural anesthesia. Given that both $CO_2$ and isoflurane cause stages of loss of consciousness, a surgical level of anesthesia, and, if used as an overdose, euthanasia,[31] the wellbeing of the animal should be the same in both situations. In other words, because the sequence of experiences for anesthesia and euthanasia are initially similar, the distress associated with induction for anesthesia or euthanasia should be the same when using the same inhalant (such as isoflurane). If the home cage does improve animal wellbeing during euthanasia, then it should also improve animal wellbeing during induction of anesthesia. However, practical reasons may underlie the use of an induction chamber for anesthesia, such as the need to anesthetize only some members of a cage cohort.

To address whether euthanasia in the home cage does in fact promote animal wellbeing as compared with use of an induction chamber, this study compared behavioral and physiologic changes of Sprague–Dawley and Brown Norway rats that were euthanized by using a 30% volume displacement per minute rate of $CO_2$ in either their home cage or an induction chamber. The physiologic responses assessed included rapid markers of activation of the HPA axis (for example, noradrenaline and blood glucose) and more delayed markers of activation of this axis (for example, corticosterone). The behavioral parameters assessed included the frequency with which each rat engaged in behaviors suggestive of anxiety and/or exploration. The hypothesis was that rats euthanized in their home cage would demonstrate fewer indices of distress than did those euthanized in the induction chamber.

## Materials and Methods

**Ethics statement.** All work described in this study was approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee prior to initiation of the study. This program is AAALAC-accredited and is compliant with all federal regulations overseeing the use of animals in research in the United States.

**Animals.** The study population comprised adult male and female Sprague–Dawley rats (24 female [age: range, 140 to 201 d; mean, 171 d]; 24 male [age: range, 114 to 167 d; mean, 137 d]), an outbred stock, and Brown Norway rats (24 female [age: range, 189 to 296 d; mean, 250 d]; 24 male [age; range, 174 to 296 d; mean, 243 d]), an inbred strain, both of which are commonly used in research.

The rats were first-generation offspring from a study evaluating the effect of cage color (clear, red, or opaque) on reproductive

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*Laboratory Animal Resource Center, School of Medicine, Indiana University, Indianapolis, Indiana*
*Corresponding author. Email: hickmand@iupui.edu*


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success. The parents (Hsd:Sprague–Dawley and BN/RijHsd) had been obtained from Envigo (Indianapolis, IN), and the rats used in this study were surplus offspring from the parent study. These rats were reared in the cage color-treatment environment until weaning at 21 d, with no other experimental manipulation performed. All animals had lived in a standard rat shoebox cage for a minimum of 98 d after weaning, prior to use in the present experiment, which was conducted under the assumption that the preweaning rearing environment would not confound the results of this study. Rats were pair-housed in IVC (Alt Design, Siloam Springs, AR) with hardwood bedding (Sani-Chip, PJ Murphy, Montville, NJ) and paper toweling for nesting materials. Food (Teklad 2018SX, Envigo, Indianapolis, IN) was provided free choice. Reverse-osmosis–treated water was provided without restriction through an automatic watering system. Cages were changed at least weekly in a laminar flow workstation (Nuaire, Plymouth, MN) and autoclaved prior to reuse. Hands and implements were disinfected with MB10 (Quip Labs, Wilmington, DE) between cages. The macroenvironment included a 12:12-h light:dark cycle (lights on, 1900), temperature of 72 ± 1 °F (22.2 ± 0.5 °C), and humidity between 30% and 70%. The colony was screened quarterly by using indirect sentinels. At the time of the study, the colony was free of the following pathogens: coronavirus (sialodacryoadenitis virus), parvoviruses (NS1, rat parvovirus type 1, Kilham rat virus, H1 virus, rat minute virus), theilovirus, *Clostridium piliforme*, *Mycoplasma pulmonis*, pinworms (*Aspicularis tetraptera*, *Syphacia* spp.), and fur mites (*Radfordia ensifer*, *Ornithonyssus bacoti*).

**Experimental design.** All rats were same-sex pair-housed, with one rat of each pair randomly assigned to the induction chamber treatment group and the other assigned to the home-cage treatment group. Because the separation of the pairs could introduce a potential confounding factor for the evaluation of stress, the experiment was designed to control for the order of euthanasia. Therefore, for half of the rat pairs, one rat was moved to the induction chamber; then the home-cage euthanasia was performed, followed immediately by the euthanasia of the rat in the induction cage. For these pairs, I hypothesized that the rats in the induction cage would experience 2 stressors (removal from familiar conspecific and placement in a novel environment) for a longer period of time, allowing for more robust physiologic changes. For the other half of the rat pairs, the rat in the induction cage was euthanized first, followed immediately by euthanasia of the rat in its home cage. For these pairs, I hypothesized that the rats in the induction cage would experience the same 2 stressors (removal from familiar conspecific and placement in a novel environment) for a shortened period of time than that experienced by pairs euthanized in the reverse order. A cage was randomly assigned to treatment group by setting up a box for each sex, with 6 'induction cage first, home cage second' and 6 'home cage first, induction chamber second' tiles in each box. After a cage was selected, a tile was drawn from the box for the appropriate sex, and the indicated treatment order was used, resulting in 2 treatment groups for each sex: (1) induction chamber rat euthanized first, home-cage rat euthanized second and (2) home cage rat euthanized first, induction chamber rat euthanized second.

**Euthanasia process.** The rats were fasted for approximately 12 to 18 h prior to euthanasia to allow measurement of fasting blood glucose values. In all cases, the induction-chamber rat was removed from the home cage and placed in an empty rat cage of the same size as the home cage but without bedding. For rats that remained in the home cage, enrichment remained, but the filter top and wire top were removed from the cage. A modified lid was placed over each cage; this lid had a port at the center for gas delivery from the top of the cage. The cage had a calculated volume of 15 L; 100% $CO_2$ was delivered from a compressed air cylinder (PraxAir, Indianapolis, IN) to the cage at a volume displacement rate of 30% per minute (approximately 5 L/min) by using a flowmeter. Rats were observed, and their behavior was scored from induction until 'nose down,' defined as the point when the head dropped and the nose of the rat touched the bottom of the cage, even if movement continued after this point. This behavioral parameter allowed approximation of the loss of righting reflex (within approximately 5 to 10 s),[16] which occurs at the end of stage 1 of anesthesia and is correlated with the loss of consciousness. Spontaneous movement can continue during stage 2 of anesthesia (from loss of righting reflex to lateral recumbency), but the animal is unconscious during that stage.[31] After a surgical plane of anesthesia (lateral recumbency, observation of regular and even respiration, confirmed by loss of response to pedal reflex) was achieved,[31] the rats were removed from their chamber, and terminal blood collection was performed through cardiac exsanguination, followed by bilateral pneumothorax to confirm euthanasia. Blood glucose was measured immediately by using a glucometer, and the remainder of the blood sample was placed in a serum separator tube, centrifuged, and then stored at –80 C until further assessment.

**Behavioral assessment.** Rats were observed from the initiation of the $CO_2$ gas delivery until nose down. The numbers of times that the rat reared (defined as raising both front feet in the air and balancing on the hindlimbs), jumped (defined as all 4 limbs leaving the floor at the same time), and engaged in digging behaviors during this period were recorded. The total number of rears, jumps, or digs was divided by the number of seconds from induction to nose down to obtain the number of rears per second, jumps per second, and digs per second (that is, the frequencies of these behaviors). These behaviors were extrapolated from published rat ethograms[5,23] as being reflective of anxiety, agitation, or escape behaviors, thus suggesting distress experienced by the rats during the euthanasia process.

**Blood glucose.** Blood glucose was measured from a whole-blood sample by using a glucometer (FreeStyle Lite; Abbott, Abbott Park, IL).

**Serum corticosterone.** Serum corticosterone was measured by using a corticosterone rat/mouse ELISA kit (07DE-9922; MP Biomedicals, Santa Ana, CA). Serum samples were undiluted. The plates were read on an ELISA plate reader set to 450 nm by using SoftMax Pro 7.0 (Molecular Devices, Sunnyvale, CA). Concentrations were calculated by using the 4-parameter logistic curve assay on MyAssays.com.

**Serum noradrenaline.** Serum noradrenaline was measured by using a noradrenaline research ELISA kit (BA E-5200; LDN Immunoassays and Services, Nordhorn, Germany). Serum samples were diluted 1:10 in 0.01 N HCl prior to processing, according to manufacturer recommendations. The plates were read on an ELISA plate reader set to 450 nm by using SoftMax Pro 7.0 (Molecular Devices). Concentrations were calculated using the 4-parameter logistic curve assay on MyAssays.com.

**Statistical analysis.** For statistical analysis, the raw data were assessed for normal distribution, followed by comparison of the means between treatments by using 2-way ANOVA. Interactions between stock or strain and treatment, stock or strain and order, sex and treatment, and sex and order (that is, induction first or second) were evaluated also. Only differences with a probability less than 0.05 were considered to be significant. All statistical analyses were conducted by using JMP 8.0 (SAS Institute, Cary, NC).


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**Table 1.** Data from Sprague–Dawley rats

| | Induction chamber | Home cage | Comment |
|---|---|---|---|
| Time to nose down (s) | 68.58 ± 1.24 | 65.63 ± 1.24 | $P = 0.0990$ |
| Blood glucose (mg/dL) | 93.21 ± 6.15 | 93.25 ± 6.15 | $P = 0.9962$<br>With treatment groups combined, blood glucose was higher ($P = 0.0149$) in males (103.54 ± 5.82 mg/dL) as compared with females (82.92 ± 5.77 mg/dL) |
| Serum corticosterone (ng/mL) | 112.56 ± 20.21<br>176.98 ± 34.57 (females)<br>49.14 ± 10.71 (males) | 85.52 ± 20.21<br>104.17 ± 34.57 (females)<br>66.86 ± 10.71 (males) | $P = 0.3489$<br>With treatment groups combined, corticosterone was lower ($P = 0.0033$) in males (58.00 ± 18.52 ng/mL) as compared with females (140.07 ± 18.52 ng/mL) |
| Noradrenaline (pg/mL) | 261.06 ± 73.44 | 197.25 ± 73.44 | $P = 0.5420$ |
| No. of jumps (per second) | 0.0011 ± 0.0007 | 0.0006 ± 0.0007 | $P = 0.6286$ |
| No. of rears (per second) | 0.116 ± 0.009 | 0.046 ± 0.009 | $P < 0.0001$ |
| No. of digs (per second) | 0 | 0 | |

All data are presented as mean ± SEM and are compared between the treatments of home cage and induction chamber. Any significant differences seen between sexes or order of treatment are presented in the Comments column.

## Results

**Overall model analysis.** In the overall model analysis, all data were compared to identify significant differences between treatments, strain and stock, and treatment order (that is, performed first compared with second) and to determine interactions between strain or stock and treatment and between treatment order and treatment.

Comparison of the mean time to nose down showed no significant difference between euthanasia in the home cage compared with the induction chamber ($F_{5,90} = 1.9942$, $P = 0.0870$), but there was a significant interaction between the strain or stock and the order of treatment ($P = 0.0162$). Mean blood glucose differed significantly between treatments ($F_{5,90} = 4.7406$, $P = 0.0007$), with a significant increase demonstrated in the Sprague Dawley rats ($P < 0.0001$). Mean serum corticosterone levels showed no overall significant treatment-associated differences ($F_{5,90} = 1.9258$, $P = 0.0977$), but there was a significant increase demonstrated in the Brown Norway rats ($P = 0.0143$). Comparison of mean serum noradrenaline levels between treatments showed a significant difference ($F_{5,90} = 18.9454$, $P < 0.0001$), with a significant increase demonstrated in the Brown Norway rats ($P < 0.0001$). Mean jump frequency showed no overall difference between treatments ($F_{5,90} = 1.2126$, $P = 0.3097$), but a significant ($P = 0.0442$) interaction between treatment and order of treatment emerged. Comparison of the mean rears frequency showed a significant difference in the overall model ($F_{5,90} = 12.8760$, $P < 0.0001$), with significant increases in the induction chamber ($P < 0.0001$) and in the Sprague Dawley rats ($P = 0.0481$). Comparison of the mean dig frequency showed a significant ($F_{5,90} = 4.1842$, $P = 0.0018$) difference in the overall model with significant increases in the home cage ($P = 0.0221$) and in the Brown Norway rats ($P = 0.0221$) and an interaction between treatment and strain or stock ($P = 0.0221$).

Given these results, the data set for each strain or stock was analyzed separately.

**Sprague–Dawley rats.** All data from Sprague–Dawley rats are presented in Table 1. Times to nose down did not differ between euthanasia in the home cage compared with induction chamber ($P = 0.1383$). Blood glucose showed no significant differences between treatment groups ($P = 0.9960$), and although there was a significant sex-associated difference, with the blood glucose of males significantly higher as compared to females ($P = 0.0162$), there was no interaction between sex and treatment ($P = 0.8159$) or between sex and order ($P = 0.3365$). Serum corticosterone did not differ between treatments ($P = 0.2950$), but levels were higher in females than males ($P = 0.0033$). However, there was no interaction between sex and treatment ($P = 0.0939$) or sex and treatment order ($P = 0.9370$). Neither serum concentration of noradrenaline ($P = 0.8391$) nor jump frequency ($P = 0.8731$) differed between treatments. The mean rear frequency was greater for the rats in the induction chamber as compared with the home cage ($P < 0.0001$). None of the Sprague–Dawley rats engaged in digging behavior, regardless of treatment, and none of the parameters showed any interaction between order of treatment and treatment.

**Brown Norway rats.** All Brown Norway data are presented in Table 2. Neither time to nose down ($P = 0.0731$), blood glucose level ($P = 0.6736$), nor jump frequency ($P = 0.3230$) differed between treatment groups. Serum noradrenaline was higher in female rats as compared with males ($P = 0.0017$), but there was no interaction between sex and treatment ($P = 0.6188$) or sex and order ($P = 0.3005$). Serum corticosterone level did not differ between treatments ($P = 0.6335$), but there was a significant sex-associated difference, with females having a higher mean level than males ($P = 0.0204$). However, there was no interaction between sex and treatment ($P = 0.4028$) or sex and order ($P = 0.7699$). The mean frequency of rears differed between treatments ($P < 0.0001$) and treatment order ($P = 0.0116$) and showed interaction between treatment order and treatment ($P = 0.0218$). Specifically, rear frequency was higher in the rats euthanized in the induction chamber as compared with the home cage, in rats euthanized first in the induction chamber as compared with second, and in rats euthanized in the home cage first as compared with second. The mean number of digs per second was higher in Brown Norway rats in the home cage as


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**Table 2.** Data from Brown Norway rats

| Treatment | Induction chamber | Home cage | Comments |
|---|---|---|---|
| Time to nose down (s) | 65.46 ± 0.80 | 67.58 ± 0.80 | $P = 0.0677$ |
| Blood glucose (mg/dL) | 71.33 ± 3.11 | 69.42 ± 3.11 | $P = 0.6650$ |
| Serum corticosterone (ng/mL) | 154.74 ± 19.36 | 142.02 ± 10.36 | $P = 0.6444$<br>With treatment groups combined, corticosterone was lower ($P = 0.0170$) in males (116.45 ± 18.23 ng/mL) as compared with females (180.32 ± 18.23 ng/mL) |
| Noradrenaline (pg/mL) | 1925.09 ± 233.05 | 1834.67 ± 233.05 | $P = 0.7850$<br>With treatment groups combined, noradrenaline was increased ($P = 0.0013$) in males (1374.16 ± 208.04 pg/mL) as compared with females (2385.60 ± 208.04 pg/mL) |
| No. of jumps (per second) | $1.08 \times 10^{?19}$ ± 0.0004 | 0.0006 ± 0.0004 | $P = 0.3225$ |
| No. of rears (per second) | 0.091 ± 0.007 | 0.038 ± 0.007 | $P < 0.0001$ |
| No. of digs (per second) | $4.34 \times 10^{?19}$ ± 0.0011 | 0.0035 ± 0.0011 | $P = 0.0262$ |

All data are presented as mean ± SEM and are compared between the treatments of home cage and induction chamber. Any significant differences seen between sexes or order of treatment are presented in the Comments column.

compared with the induction chamber ($P = 0.0239$). Except for rearing, there was no significant interaction between treatment order and treatment in any parameter.

## Discussion

Sprague–Dawley and Brown Norway rats showed no significant differences in the selected neurophysiologic measurements between induction chamber and home cage euthanasia, regardless of the order of the euthanasia. The sympathetic–adrenal–medullary system likely is rapidly stimulated due to exposure to $CO_2$,[10,14,19,22] but there are no significant differences dependent upon whether the rats experience euthanasia in either the home cage or induction chamber.[17,32] Likewise, a mean time to nose down of approximately 1 min may provide sufficient time for alterations in circulating blood glucose in response to the sympathetic–adrenal–medullary system and HPA axis, but that effect may require additional time to reach significant differences.[18] The sex-associated differences observed in the serum corticosterone and noradrenaline levels for rats in the current study are consistent with the existing literature,[3,9] but the significant increases in the measured levels of noradrenaline in the Brown Norway rats were unexpected. However, mean ventilatory sensitivity differs between Brown Norway and Sprague–Dawley rats, suggesting that the observed difference in noradrenaline could be due to the response to the hypercapnia induced by $CO_2$.[12,27,28] Concurrent evaluation of the interactions between ventilatory sensitivity and noradrenaline levels is an area that would benefit from additional exploration.

Significant sex-associated differences in blood glucose level were not expected, but the Sprague-Dawley males in this study exhibited a significant increase in this parameter. The male rats may not have consistently been completely fasted, highlighting one of the concerns regarding using a measurement that fluctuates with time of day and fasted state. Although the use of neuroendocrine hormones has been recommended as being more sensitive than corticosterone and blood glucose,[4] caution should be taken because the use of $CO_2$ induces a respiratory acidosis that causes the amygdala to release of the neuroendocrine hormone.[29,30,33] Because samples were collected after an animal was unconscious, the observed changes in noradrenaline might be due to the response to respiratory acidosis and thus do not reflect distress during induction.[11] For this reason, the behavioral data can provide additional information.

For both types of rats, minimal jumping behavior was noted and did not differ significantly between treatment groups. Brown Norway rats engaged in rare digging behavior in the home cage, but the Sprague–Dawley rats did not. Neither Brown Norway nor Sprague–Dawley rats displayed digging behavior in the induction chamber, whereas both populations demonstrated significantly more rearing behavior when in the induction chamber as compared with the home cage. The observed rearing appeared to be consistent with the exploratory behavior associated with a new space. The absence of jumping and digging behavior seems to support the conclusion that the rats did not experience increased agitation associated with $CO_2$ in the induction chamber. In addition, approximately 10% of rats repeatedly returned to the port and sniffed the 100% $CO_2$ that was being introduced into the cage, despite reports by humans that inhalation of 100% $CO_2$ is extremely painful.[7] However, in that oft-referenced study of pain associated with inhalation of $CO_2$ by humans,[7] 13 of the 40 participants rated 80% $CO_2$ as uncomfortable (not painful), and 2 of the 40 rated 100% $CO_2$ as uncomfortable (not painful), thus indicating definite interindividual differences associated with tolerance of exposure to high concentrations of $CO_2$.

If keeping a rat in its home cage to minimize the stress associated with the induction phase of euthanasia when using an inhalant anesthetic, it follows that the home cage should also be used to perform inhalational anesthesia. This study demonstrated that despite several strain-specific differences in responses to the process of euthanasia, there were generally no significant differences in these responses between the treatment groups of home cage compared with induction chamber. These findings suggest that—from the perspective of a rat—either the home cage or an induction chamber can be used for the


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induction of anesthesia using $CO_2$, regardless of whether the intended purpose of the induction is for anesthesia prior to a procedure or for euthanasia.

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